# IL-2 regulates expansion of CD4$^+$ T cell populations by affecting cell death: insights from modeling CFSE data: Supplementary Material

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February 18, 2007

## 1 Appendix I: additional results of fittings

### 1.1 Dynamics of undivided cells: analysis of data from the thymidine labeling experiments (TLE)

Naive T cells stimulated in vitro or in vivo typically undergo their first division at much slower rate than their subsequent divisions (1–5). Deenick et al. (1) have developed a novel approach to obtain an insight into the initial dynamics of naive T cells after in vitro stimulation. The time taken by different naive CD4$^+$ T cells to progress to the S-phase after anti-CD3 stimulation was estimated by measuring incorporation of thymidine ([$^3$H]TdR) by cells after a short pulse of the radioactive label (see main text and Figure 2).

Previously these data have been fitted by a lognormal distribution with 3 parameters: the relative number of cells recruited into the S-phase of the cell cycle (i.e., area under the theoretical curve), the average time to the S-phase, and its variance (1). We refitted these data using gamma and lognormal distributions with an added delay (see main text and Figure 2). Since there might be a

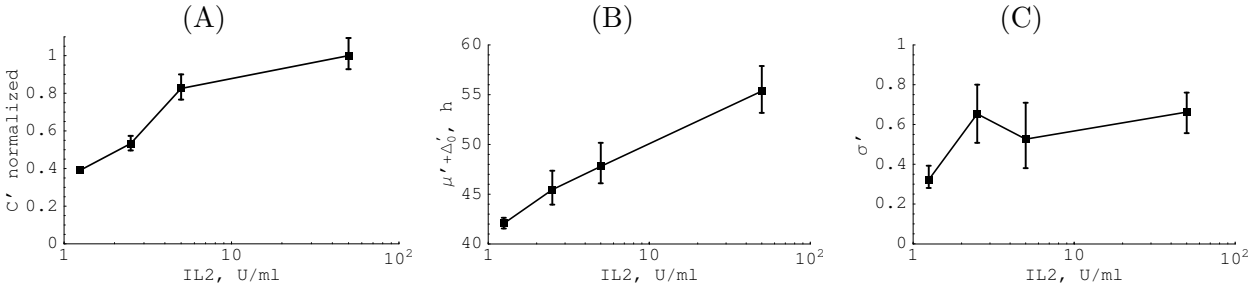


**Figure 1:** Changes in parameters, determining recruitment of naive CD4$^+$ T cells into the first S-phase of the cell cycle, with the IL-2 concentration. Panel A: fraction of cells recruited into the S-phase $C'$ (i.e., the relative area under the curve normalized to that of IL-2=50 U/ml). Panel B: the average time to the S-phase of surviving cells $\mu' + \Delta'_0$, where $\mu'$ is the mean of the lognormal distribution and $\Delta'_0$ is a delay. Panel C: the shape parameter of the lognormal distribution $\sigma'$.

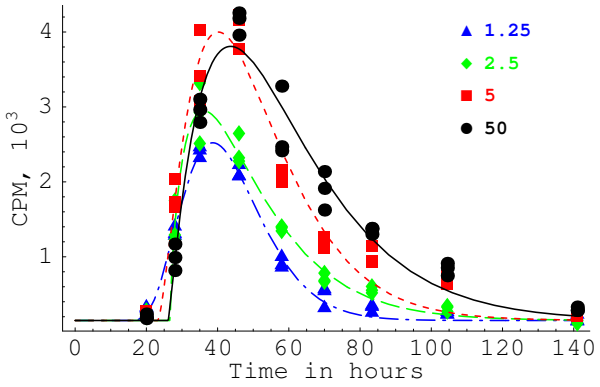


**Figure 2:** Investigating the influence of IL-2 on the recruitment of undivided cells into the S-phase of the cell cycle. Gamma distribution with a delay was fitted to the TLE data. Introduction of a delay led to a significant improvement of the quality of fits at all IL-2 concentrations ($p < 0.01$, F-test for nested models). Fixing the shape parameter $k = 2$ led to a significantly lower quality of the fit only at IL-2=1.25 U/ml ($p < 0.05$, F-test). Parameters for the fits are given in Table 1.

| | | **IL-2=1.25 U/ml** | | **IL-2=2.5 U/ml** | | **IL-2=5 U/ml** | | **IL-2=50 U/ml** | |
|---|---|---|---|---|---|---|---|---|---|
| | | mean | 95% CIs | mean | 95% CIs | mean | 95% CIs | mean | 95% CIs |
| $\mu'$ | h | 29.7 | 25.77–34.85 | 22.81 | 21.17–25.1 | 27.64 | 25.4–34.47 | 33.1 | 30.8–36.23 |
| $\sigma'$ | h | 12 | 11.37–12.77 | 17.47 | 15.14–20.79 | 17.12 | 14.68–20.16 | 22.71 | 19.64–26.46 |
| $\Delta'_0$ | h | 13.8 | 7.91–18.2 | 26.7 | 24.38–27.85 | 23.15 | 14.34–26.32 | 26.14 | 22.96–27.36 |
| $C', 10^4$ | cpm | 6.64 | 6.4–6.89 | 8.82 | 8.35–9.51 | 13.65 | 12.71–14.88 | 16.29 | 15.16–17.6 |

**Table 1:** Parameter estimates with 95% confidence intervals (CI) obtained by fitting gamma distribution with a delay to the TLE data. Here $\mu'$, $\sigma'$, and $\Delta'_0$ being mean, standard deviation, and a delay for the distribution, respectively, and $C'$ is the total area under the curve. Confidence intervals have been obtained by bootstrapping the residuals with 500 simulations.

minimal time taken by cells to progress to the S-phase of the cell cycle, such a delay seems biologically reasonable. Importantly, introduction of a delay in both distributions led to a significant increase in the quality of the fit for most IL-2 concentrations ($p > 0.05$ only for IL-2=1.25 U/ml for lognormal distribution, $p < 0.05$ for all other IL-2 concentrations for both lognormal and gamma distribution; F-test for nested models). Since a lognormal distribution allowed for a better fit of the data than the gamma distribution at all IL-2 concentrations as judged by the mean square (which is the sum of squared residuals divided by the degrees of freedom), further analysis shown in the main text was done with the lognormal distribution. For the lognormal distribution, we find that the fraction of of cells recruited into the response and survived until the S-phase increases with the increasing IL-2 concentration (Figure 1A). However, in contrast with previous results of Deenick et al. (1) we find that the average time to the S-phase for such cells increases with the increasing IL-2 concentration (Figure 1B), which seems unexpected. The shape parameter $\sigma'$ hardly changes at high IL-2 concentrations and is the lowest at the lowest IL-2 concentration, leading to a distribution with the smallest variance at IL-2=1.25 U/ml. These results are qualitatively similar to those obtained with a gamma distribution (see Figure 2 and 3, and Table 1). Overall, these data suggest that IL-2 increases the number of T cells recruited, and slows down recruitment into the first S-phase after stimulation.

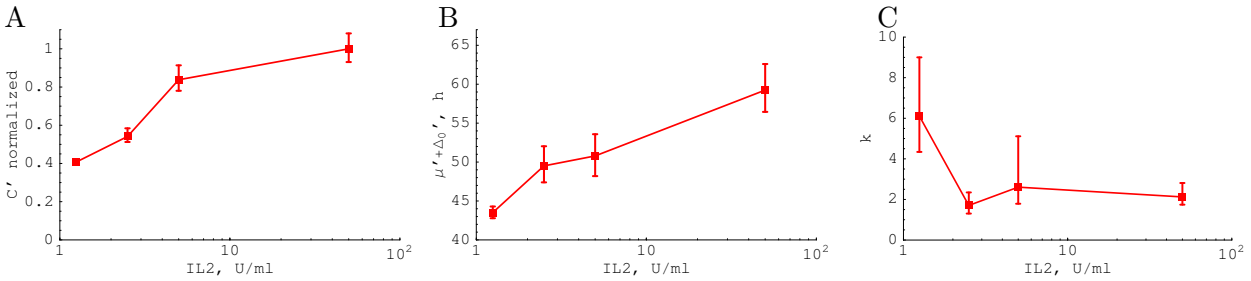


**Figure 3:** Changes of estimated parameters with the IL-2 concentration for fits shown in Figure 2: a fraction of cells recruited into the S-phase (i.e., the relative area under the curve, panel A), and the average time to the S-phase of surviving cells $\mu + \Delta_0$ (panel B), the shape parameter $k$ (panel C). Interestingly, the shape of the gamma distribution, characterized by the shape parameter $k$, does not change at high IL-2 concentration (IL-2 $\geq$ 2.5 U/ml) with $k \approx 2$ and is higher at IL-2=1.25 U/ml ($k \approx 6$). The observation that the shape parameter of the gamma distribution is close to 2 is particularly interesting because it suggests that recruitment of undivided $CD4^+$ T cells into the first S-phase may represent a two-step process with exponential (identical) waiting times. Biologically, these two steps can be two transitions cells make following stimulation with antigen: transition from G0 to G1-phase, and from G1 to S-phase (6).

# 2 Appendix II: mathematical derivations

## 2.1 Recruitment function $R(t)$

In most fittings, recruitement function $R(t)$ is defined as a lognormal distribution (given in the main text). In some other cases, we define the recruitment function $R(t)$ as a gamma distribution

$$R(t) = \begin{cases} \frac{C\theta^k (t-\Delta_0)^{k-1}}{\Gamma(k)} e^{-\theta(t-\Delta_0)}, & \text{if } t \geq \Delta_0, \\ 0, & \text{otherwise.} \end{cases} \tag{1}$$

where $\theta$ and $k$ are the rate and shape parameters respectively, $C$ is the constant, $\Delta_0$ is the delay, and $\Gamma(\cdot)$ is the Euler gamma function. In this distribution, mean and standard deviation can be easily calculated, $\mu = k/\theta$ and $\sigma = \sqrt{k}/\theta$. In fittings of the TLE data we have set a background level of radioactivity of unlabeled cultures being 150 cpm (P. Hodgkin, personal communication).

## 2.2 Smith-Martin model

We reformulate the SM model of cell division and death proposed in earlier papers (7, 8) to include the recruitment function $R(t)$ (which is the number of cells entering the second division class per unit of time). The dynamics of the number of cells in the A-state and the time density of cells in the B-phase of age $\tau$ undergone $n$ divisions by time $t$ ($A_n(t)$ and $b_n(t,\tau)$, respectively) are given by equations

$$\frac{\mathrm{d}A_1(t)}{\mathrm{d}t} = R(t) - (\lambda + d)A_1(t), \tag{2}$$

$$\frac{dA_n(t)}{\mathrm{d}t} = 2b_{n-1}(t,\Delta) - (\lambda + d)A_n(t), \qquad n > 1, \tag{3}$$

$$\frac{\partial b_n(t,\tau)}{\partial t} + \frac{\partial b_n(t,\tau)}{\partial \tau} = -d b_n(t,\tau), \qquad n \geq 1 \quad 0 \leq \tau \leq \Delta, \tag{4}$$

where $\lambda$ is the rate at which cells leave the A-state, $\Delta$ is the duration of the B-phase, $d$ is the cell death rate (which is assumed to be same in the A-state and the B-phase). The initial and boundary conditions for the model are: $A_n(0) = b_n(0,\tau) = 0$, $b_n(t,0) = \lambda A_n(t)$. Solving eqn. (4) using method of characteristics with the given boundary condition yields

$$b_n(t,\tau) = \lambda A_n(t-\tau)e^{-d\tau}. \tag{5}$$

where $B_n(t) = \int_0^{\Delta} b_n(t,\tau)\, d\tau$ is the total number of cells in the B-phase undergone $n$ divisions by time $t$. The dynamics of cells in the B-phase of the cell cycle undergone one division can be found by integrating eqn. (4) in $\tau$

$$\frac{\mathrm{d}B_1(t)}{\mathrm{d}t} = \int_0^{\Delta} b_1(t,\tau)\, d\tau = \lambda A_1(t) - \lambda e^{-d\Delta} A_1(t-\Delta) - dB_1(t). \tag{6}$$

To derive explicit formulas for the dynamics of the total number of cells undergone $n$ divisions for $n > 1$, we take the first derivative of $B_n(t)$ and then substitute $A_n'(t-\tau)$ with the expression given in eqn. (3) and (5):

$$\begin{aligned} \frac{\mathrm{d}B_n(t)}{\mathrm{d}t} &= \lambda \int_0^{\Delta} A_n'(t-\tau)e^{-d\tau}\, d\tau = 2\lambda^2 e^{-d\Delta} \int_0^{\Delta} A_n(t-\Delta-\tau)e^{-d\tau}\, d\tau \\ &- \lambda(\lambda+d) \int_0^{\Delta} A_n(t-\tau)e^{-d\tau}\, d\tau = 2\lambda e^{-d\Delta} B_{n-1}(t-\Delta) - (\lambda+d)B_n(t) \quad n > 1. \end{aligned} \tag{7}$$

By summing the eqns. (3) and (7), we find that the dynamics of the total number of cells undergone $n$ divisions $X_n(t) = A_n(t) + B_n(t)$ is governed by the equation:

$$\frac{\mathrm{d}X_n(t)}{\mathrm{d}t} = 2\lambda e^{-d\Delta} X_{n-1}(t-\Delta) - (\lambda+d)X_n(t), \qquad n \geq 2. \tag{8}$$

This is an important result because it suggests that the dynamics of the total number of cells in the SM model for $n > 1$ can be determined by solving eqn. (8). Thus, eqns. (2), (6), and (8) can be used to obtain the numerical solution of the SM model.

In some instances it may be useful to write the solution of eqn. (8). For that we apply Laplace transformation to eqn. (8):

$$\mathcal{L}\{X_n(t)\} = \left(\frac{2\lambda e^{-(s+d_B)\Delta}}{s+\lambda+d_A}\right) \mathcal{L}\{X_{n-1}(t)\},\ n \geq 2, \tag{9}$$

or simply that

$$\mathcal{L}\{X_n(t)\} = \left(\frac{2\lambda e^{-(s+d_B)\Delta}}{s+\lambda+d_A}\right)^{n-1} \mathcal{L}\{X_1(t)\},\ n \geq 1. \tag{10}$$

Inverting the Laplace transform given in eqn. (10), we find

$$X_n(t) = \frac{(2\lambda e^{(d+\lambda-d)\Delta})^{n-1}}{(n-2)!} \int_{(n-1)\Delta}^{t} (\tau-(n-1)\Delta)^{n-2} e^{-(\lambda+d)\tau} X_1(t-\tau)\, d\tau, \quad n \geq 2 \tag{11}$$

If the parameters of cell division and death depend on time or on the number of divisions cells have undergone, the model (2)–(4) has to be used for simulations. The model can be rewritten as a system of delayed differential equations.

**Parameters depend on time**. First, we solve eqn. (4) by the method of characteristics taking into account the initial and boundary conditions $A_n(0) = b_n(0,\tau) = 0$ and $b_n(t,0) = \lambda(t)A_n(t)$. The solution is

$$b_n(t,\tau) = \lambda(t-\tau)A_n(t-\tau)e^{-\int_{t-\tau}^{t} d(s)\mathrm{d}s}. \tag{12}$$

By definition, the number of cells in the B-phase undergone $n$ divisions by time $t$ is $B_n(t) = \int_0^{\Delta(t)} b_n(t,\tau)\mathrm{d}\tau$. Differentiating $B_n(t)$ in time yields

$$\frac{\mathrm{d}B_n(t)}{\mathrm{d}t} = \int_0^{\Delta(t)} \frac{\partial b_n(t,\tau)}{\partial t}\mathrm{d}\tau + b_n(t,\Delta(t))\Delta'(t). \tag{13}$$

On the other hand, after integrating eqn. (4) in $\tau$, we find

$$\int_0^{\Delta(t)} \frac{\partial b_n(t,\tau)}{\partial t}\mathrm{d}\tau + b_n(t,\Delta(t)) - b_n(t,0) = -d(t)B_n(t), \tag{14}$$

Combining eqns. (13) and (14), we arrive at the equations describing the dynamics of cells in the B-phase of the cell cycle, resulting in the following system:

$$\frac{\mathrm{d}A_1(t)}{\mathrm{d}t} = R(t) - (\lambda(t)+d(t))A_1(t), \tag{15}$$

$$\frac{\mathrm{d}A_n(t)}{\mathrm{d}t} = 2\lambda(t-\Delta(t))e^{-\int_{t-\Delta(t)}^{t} d(s)\mathrm{d}s} A_{n-1}(t-\Delta(t)) - (\lambda(t)+d(t))A_n(t). \qquad n>1, \tag{16}$$

$$\frac{\mathrm{d}B_n(t)}{\mathrm{d}t} = \lambda(t)A_n(t) - \lambda(t-\Delta(t))A_n(t-\Delta(t))e^{-\int_{t-\Delta(t)}^{t} d(s)\mathrm{d}s}(1-\mathrm{d}\Delta(t)/\mathrm{d}t) - d(t)B_n(t), \qquad n \geq 1 \tag{17}$$

**Parameters depend on the division number**. Proceeding similarly as in the SM model with time-dependent parameters, we arrive at the following formulation

$$\begin{aligned}
\frac{\mathrm{d}A_1(t)}{\mathrm{d}t} &= R(t) - (\lambda_1 + d_1)A_1(t), && (18)\\
\frac{\mathrm{d}A_n(t)}{\mathrm{d}t} &= 2\lambda_{n-1}e^{-d_{n-1}\Delta_{n-1}}A_{n-1}(t-\Delta_{n-1}) - (\lambda_n + d_n)A_n(t). \qquad n > 1, && (19)\\
\frac{\mathrm{d}B_n(t)}{\mathrm{d}t} &= \lambda_n A_n(t) - \lambda_n e^{-d_n\Delta_n}A_n(t-\Delta_n) - d_n B_n(t), \qquad n \geq 1 && (20)
\end{aligned}$$

where $\lambda_n$, $\Delta_n$ and $d_n$ are the commitment rate, the duration of the deterministic phase, and the death rate, respectively, dependent on the division number $n$. The model can be easily extended with different death rates in the A-state and the B-phase.

### 2.3 IL-2 consumption model

In the model we assume that cells consume IL-2 at first and later divisions proportional to the density of cells and rate of division of undivided cells, given by the recruitment function $R(t)$:

$$\frac{\mathrm{d}I(t)}{\mathrm{d}t} = -\Big[c_1 X(t) + c_2 R(t)\Big]\frac{I(t)}{h_I + I(t)}, \tag{21}$$

where $I(t)$ is the concentration of IL-2 at time $t$ after stimulation, and $X(t) = \sum_{n=1}^{\infty} X_n(t)$ is the total number of live cells in the culture. Note that we use a saturating function to describe consumption of IL-2. A simple linear term does not yield satisfactory description of the data (results not shown). Parameters for cell division and death in the SM are assumed to depend on the current IL-2 concentration $I$ in a simple saturating form:

$$\begin{aligned}
\lambda(I) &= \lambda_1 + \frac{(\lambda_2 - \lambda_1)I}{h_\lambda + I}, && (22)\\
\Delta(I) &= \Delta_1 + \frac{(\Delta_2 - \Delta_1)I}{h_\Delta + I}, && (23)\\
d(I) &= d_1 + \frac{(d_2 - d_1)I}{h_d + I}. && (24)
\end{aligned}$$

Note that this description allows parameters $\lambda$, $\Delta$, and $d$ to decrease, increase or remain constant with increases of the IL-2 concentration. One could solve numerically the original SM model given in eqns. (2)–(4) with parameters dependent on the current IL-2 concentration. For numerical reasons, we have found it more convenient to rewrite the model as a system of delay differential equations. This is done similarly to the derivation of the model with time-dependent parameters, and the resulting model takes the following form

$$\frac{\mathrm{d}A_1(t)}{\mathrm{d}t} = R(t) - (\lambda[I(t)] + d[I(t)])A_1(t), \tag{25}$$

$$\frac{\mathrm{d}A_n(t)}{\mathrm{d}t} = 2\lambda[I(t-\Delta[I(t)])]e^{-\int_{t-\Delta[I(t)]}^{t} d[I(s)]\mathrm{d}s} A_{n-1}(t-\Delta[I(t)]) - (\lambda[I(t)] + d[I(t)])A_n(t), \quad n > 1, \tag{26}$$

$$\frac{\mathrm{d}B_n(t)}{\mathrm{d}t} = \lambda[I(t)]A_n(t) - \lambda[I(t-\Delta[I(t)])]e^{-\int_{t-\Delta[I(t)]}^{t} d[I(s)]\mathrm{d}s} A_{n-1}(t-\Delta[I(t)]) \times \left[1 - \frac{\partial\Delta}{\partial I}\frac{\mathrm{d}I(t)}{\mathrm{d}t}\right] - d[I(t)]B_n(t), \quad n \geq 1. \tag{27}$$

There are several assumptions put in this formulation of the model. For example, we assume that the duration of the B-phase, $\Delta[I(t)]$, is determined by the IL-2 concentration at the end of B-phase, i.e. $\Delta = \Delta[I(t)]$. Other possibilities are more difficult to include in this formulation of the model. For example, it is difficult to let the decision on the length of the B-phase is made in the beginning of the B-phase given the current IL-2 concentration. Also, we assume that the recruitment function $R(t)$ does not explicitly depend on the current IL-2 concentration, but only on the initial IL-2 concentration via the parameters estimated from the TLE and in fitting CFSE data. An alternative model could include explicit description of the dynamics of undivided cells with paramters, governing these dynamics, being dependent on the IL-2 concentration.

# 3 Additional figures for the paper

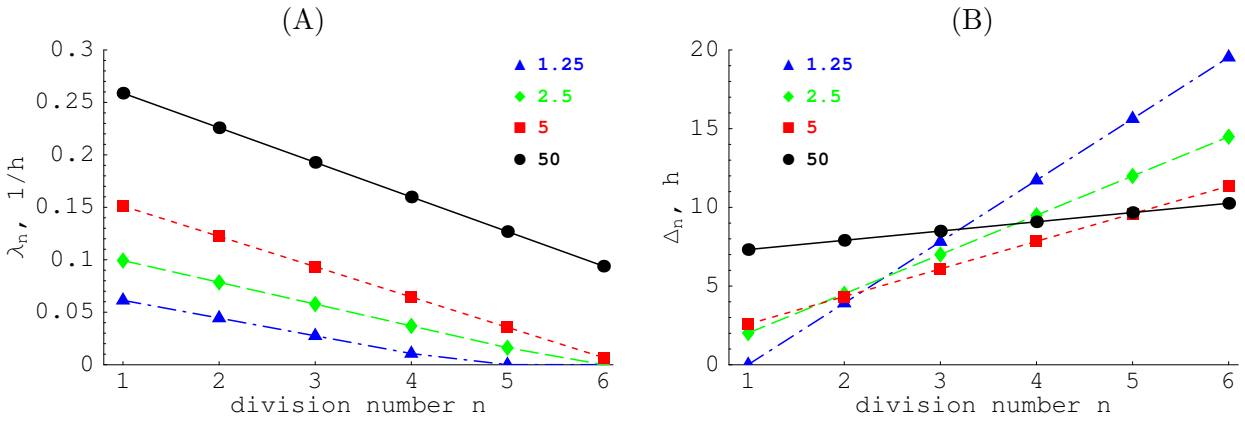


**Figure 4:** Estimated changes in the rate of commitment to division $\lambda_n$ (panel A) or in the duration of the B-phase $\Delta_n$ (panel B) with the division number $n$ (panel B). We fitted the SM model to the CFSE data assuming either $\lambda_n = \lambda_1 + \alpha(n-1)$ (panel A) or $\Delta_n = \Delta_1 + \alpha(n-1)$ (panel B). Quality of the model fits was similar (based on sum of squared residuals) to those in the main text with the death rate $d_n$ changing linearly with the division number.

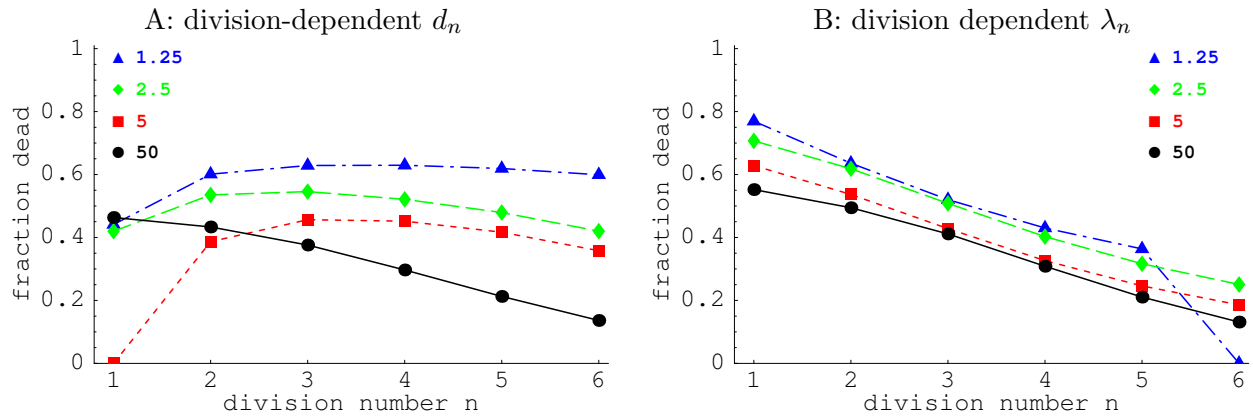


**Figure 5:** Predicted fractions of dead cells in each division class at 96 hours after stimulation in a model with division-dependent death rate $d_n$ (panel A) and division-dependent committment rate to division $\lambda_n$ (panel B). To model the dynamics of dead cells undergone $n$ divisions $D_n$ we used the following equation: $\mathrm{d}D_n(t)/\mathrm{d}t = d_n(A_n(t) + B_n(t)) - d_D D_n(t)$, where $d_D$ is the rate of dead cell disintegration. In general, disintegration rate of cells in vitro is unknown, and most likely it depends on the cell type and medium conditions. We used an estimate $d_D = 0.05/h$ found previously for lymphocytes (9), but a range of disintegration rates gave similar results. The model with division-dependent death rate predicts different change in the fraction of dead cells with cell division number for IL-2=50 and 1.25 U/ml (higher fractions of dead cells in later divisions for the lowest IL-2 concentration). The model with division-dependent committment rate predicts a similar decrease in the fraction of dead cells with division number for all IL-2 concentrations.

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